

A preliminary study on antibacterial efficacy of the methanolic extract of *acalypha wilkesiana* leaves against selected clinical isolates.

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ABSTRACT

The methanolic leaves extract of *A. wilkesiana* was investigated for antibacterial activities and preliminary phytochemical screening. The phytochemical screening was carried out using standard procedures while the antibacterial susceptibility was done using the agar well diffusion method. The phytochemical screening result showed the presence of tannins, saponins, flavonoids, cardiac glycosides, alkaloids, steroids as well as terpenoids. The antibacterial susceptibility showed zones of inhibition that ranged between 4mm and 13mm. The MIC ranged between 10mg/ml and 30mg/ml while the MBC range between 30mg/ml and 50mg/ml, for all the organisms used. The results obtained from this research gave scientific support to the use of *A. wilkesiana* leaves for bacterial chemotherapy and it is suggested that the plant extract could be a promising lead compound for the synthesis of effective chemotherapeutics against the diseases caused by the test organisms used.

Keywords: Antibacterial, methanolic,, phytochemical, extract.

INTRODUCTION

The use of plant, plant extract or plant derived chemicals to treat disease; topical, subcutaneous and systemic, has stood the test of time (Oladunmoye, 2006). In recent years, there has been a gradual revival of interest in the use of medicinal plant in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Iniaghe *et al.*, 2009). More importantly in Africa, particularly West Africa, new drugs are often beyond the reach of the poor such that up to 80% of the population use medicinal plant as remedy against infections and disease (Hostettman and Maston, 2002).

Herbal drugs are finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations (WHO, 1991). The use of herbal medicines has greatly increased in line with the global trend of people returning to natural therapies. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription (Choudhary and Sekhon, 2011).

According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens.

From history, mankind has used plants (herbs) or herbal medicine in attempt to cure disease and relieve suffering. Current estimate suggest that, in many developing countries, a large proportion of the population rely heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicines may be available in these countries, herbal medicine (phytomedicine) has often maintained popularity for historical and cultural reason (Zhang, 1999).

Acalypha wilkesiana Muell Arg (copper leaf) is a plant from the family Euphorbiaceae. The genus *Acalypha* comprises about 570 species (Riley, 1963), a large proportion of which are weeds while the others are ornamental plants. The plants are found all-over the world especially in the tropics of Africa, America and Asia. The weeds are wild and can be found growing everywhere, while the ornamental species must have been introduced into West Africa from other parts of the world and are cultivated as foliage plants in gardens and greenhouses. It is a fast growing evergreen shrubs which provides a splash of colour in the landscape with bronze red to muted red, the leaves appear as heart shaped with combination of colour like green, purple, yellow, orange, pink or white depending on cultivation (Madziga *et al.*, 2010). Its other names include; *A. amentaceae* and *A. tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, beef steak plant and match-me-if-you-can (Christman, 2004). The Hausas of Northern Nigeria call it "Jiwene" and "Jinwinini", while the Yoruba of Southern Nigeria call it "aworoso" (Christman, 2004). *A. wilkesiana* is native to Fiji and nearby islands in the South Pacific, and it is a popular outdoor plant that provides colour throughout the year, although it is also grown indoors as a container plant. It is propagated by stem cuttings at any time of the year.

MATERIALS AND METHODS

Hot air oven (model: GP/50/CLAD/250/HYD) (serial no.08G010)

- Electronic Weighing Balance
- Blender (Philip, Japan)
- Mueller Hutton Agar: Titan Biotech Ltd. Bhiwadi – 301019, Rajasthan India
- Methanol: (BDH) Chemical Ltd, Poole, England
- Dragendorff Reagent
- Meyer's Reagent
- Wagner's reagent
- Hager's reagent
- Concentrated Hydrochloric acid
- Dimethyl sulphoxide
- Concentrated tetraoxosulphate (VI) acid
- Chloroform
- Savlon

Collection of Plant Materials

Fresh leaves of *A. wilkesiana* were obtained from the University herbarium, Delta State University, Abraka and identified in the department of Pharmacognosy, faculty of Pharmacy, Delta State University, Abraka. The leaves were washed with tap water, dried in the oven at 40°C and then pulverized using blender. This was then stored in a sealed bag until it was used.

Extraction Procedures:

120 grams of oven-dried powdered leaves was added to one litre of 70% methanol in a glass bottle. The sample was kept in the dark for 7 days with intermittent shaking. The percolates were filtered with Whatmans No 1 filter paper and the filtrate was then concentrated at 40°C and the final product was stored in a universal bottle at 4°C prior to testing.

Source of Test Organism:

The test organisms were isolates from the Delta State University Teaching Hospital Oghara. The organisms are: *Staphylococcus aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and these bacteria were confirmed using standard biochemical test, motility test, microscopy and culture plate techniques and these served as the test bacteria.

Phytochemical Analysis

Phytochemical screening were performed on the plant extract to determine the presence of active chemical constituents in the leaves of *A. wilkesiana* using standard method employed Saranraj *et al.* (2010) and Oluduro *et al.* (2011). The plant extract was screened for the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides and terpenoids.

Standardization of Bacteria Inoculum

All the test organisms were sub-cultured on nutrient agar for 24 hours and few colonies were transferred into 5ml of sterile nutrient broth in test tubes and incubated for 30 minutes at 37°C. The growth of bacterial suspension obtained was adjusted to 0.5 McFarland standard (10^6 cfu/ml).

Determination of Antimicrobial Activity

The antibacterial activities of the extract of the leaves were determined using agar well diffusion technique (Chen *et al.*, 1997). The well was bored with a sterile cork borer (5mm in diameter) on the already set Mueller Hinton agar plate. After which, the Muller Hilton agar plates were aseptically flooded with the standardized bacterial culture 6 (1.5×10^6 cfu/ml). Ciprofloxacin was used as the control.

0.1ml of various concentrations of the extract was introduced into the holes using sterilized syringes with needles. Four plates of Muller Hutton agar were prepared and inoculated with the test bacteria and the sensitivity disc of Ciprofloxacin was placed on the plates as control. The inoculated Petri dishes were left for a few minutes for the extract to diffuse into the agar. The plates were then incubated at 37°C for 24 hours. The zones of inhibition were measured using a measuring millimetre rule.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined by agar diffusion technique. 1ml of 10mg/ml of the extract was mixed with 19ml of molten Mueller Hinton agar and swayed gently before allowing it to solidify. This procedure was repeated for 20mg/ml, 30mg/ml, 40mg/ml, 50mg/ml, 60mg/ml, 70mg/ml, 80mg/ml, 90mg/ml and 100mg/ml and the test organisms were streaked on the agar and incubated for 24 hours at 37°C. The MIC was taken as the least concentration that inhibited the growth of the test organisms. MBC was determined by sub-culturing the test

organism from MIC test onto fresh solid medium and incubated for 24 hours. The lowest concentration in which no growth occurred was taken as the MBC.

Results

Table 1. Preliminary Phytochemical Analysis of Leaves of *Acalypha wilkesiana*.

| SECONDARY METABOLITES | PLANT | METHANOLIC EXTRACT | LEAF | INFERENCES |
|-----------------------|-------|--------------------|------|-------------------------|
| Alkaloids | | + | | Present in trace amount |
| Flavonoids | | +++ | | Highly Present |
| Saponins | | ++ | | Moderately Present |
| Tannins | | +++ | | Highly Present |
| Steroids | | + | | Present in trace amount |
| Cardiac Glycosides | | + | | Present in trace amount |
| Terpinoids | | + | | Present in trace amount |

Table 2. Minimum Inhibitory Concentration

| Organisms | 10mg/ml | 20mg/ml | 30mg/ml | 40mg/ml | 50mg/ml | 60mg/ml | 70mg/ml | 80mg/ml | 90mg/ml | 100mg/ml |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|
| <i>E. coli</i> | NG | NG | G | G | NG | G | NG | NG | NG | NG |
| <i>K. pneumonia</i> | G | G | NG | NG | NG | NG | G | NG | NG | NG |
| <i>S. aureus</i> | NG | G | G | G | NG | NG | NG | NG | NG | NG |
| <i>P. aeruginosa</i> | G | G | NG | NG | NG | NG | G | NG | NG | NG |

NG = No Growth

G = Growth

Table 3. Minimum Bacteriocidal Concentration

| Organisms | MBC(mg/ml) |
|----------------------|------------|
| <i>E. coli</i> | 50 |
| <i>K. pneumonia</i> | 30 |
| <i>S. aureus</i> | 50 |
| <i>P. aeruginosa</i> | 30 |

Table 4a. Zone of Inhibition of Leaves Extract

| Organisms | 10mg/ml | 20mg/ml | 30mg/ml | 40mg/ml | 50mg/ml | 60mg/ml | 70mg/ml | 80mg/ml | 90mg/ml | 100mg/ml |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|
| <i>E. coli</i> | 10mm | - | - | - | 9mm | - | 6mm | - | 10mm | 15mm |
| <i>K. pneumonia</i> | - | - | 10mm | - | 12mm | 6mm | - | - | 13mm | 10mm |
| <i>S. aureus</i> | 4mm | - | - | - | 7mm | - | 10mm | - | 10mm | 13mm |
| <i>P. aeruginosa</i> | - | - | 10mm | - | 5mm | 6mm | - | - | 13mm | 13mm |

Table 4b. Zone of Inhibition of the Control Antibiotic

| Organisms | Ciprofloxacin |
|----------------------|---------------|
| <i>E. coli</i> | 44mm |
| <i>K. pneumonia</i> | 34mm |
| <i>S. aureus</i> | 40mm |
| <i>P. aeruginosa</i> | 38mm |

The percentage yield of the extract was 15%. The phytochemical testing of the extract gave positive reactions for alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides and terpenoids as shown on Table 1. The Minimum Inhibitory Concentration (MIC) of the extract for *E. coli* and *S. aureus* was 10mg/ml while that of *K. pneumonia* and *P. aeruginosa* was 30mg/ml as shown in Table 2. The minimum Bactericidal Concentration (MBC) of the extract for *K. pneumonia* and *P. aeruginosa* was 30mg/ml, the MBC for *E. coli* and *S. aureus* was 50mg/ml as shown in table 3.

The extract inhibited the growth of all the organisms tested in varying degrees at various concentrations as indicated by their zones of inhibition in Table 4. *E. coli* showed zone of inhibition of 15mm at the highest concentration of extract tested (100mg/mL), *S. aureus* showed zone of inhibition of 13mm also at the highest concentration of the extract tested (100mg/mL), *P. aeruginosa* showed zone of inhibition of 13mm at the two highest concentration of extract tested (90mg/ml and 100mg/mL) while *K. pneumonia* showed zone of inhibition of 13mm at a concentration of 90mg/mL.

Discussion

18g of the leaf extract (15%) was obtained from the dried powder of leaves (120g). 70% methanol (Methanolic extraction) was used to enable extract components in both the aqueous and the methanolic phase and the considerable high yield (18g) of the extract may be due to extraction in both phases as water soluble components could be soluble in the aqueous phase and components soluble in methanol, in the methanolic phase.

The phytochemical analysis of the leaf extract showed the presence of some Secondary Plant Metabolites in varying degree as shown in Table 1. Tannins and Flavonoids were seen to be highly present, Saponin was seen to be moderately present while Alkaloids, Cardiac glycosides, Terpenoids and Steroids were seen to be present in trace amount. The presence of these phytochemicals in the leaf extract, shows that the extract possess anti-microbial properties which are responsible for its potent activity against bacteria.

The leaves extract showed antimicrobial activities on the test organisms in varying degree. The entire test Organisms were susceptible to the leaves extract but not at all concentrations used. They all showed resistance at three different concentrations out of the ten concentrations used. *E.coli* showed resistance at 30mg/ml, 40mg/ml and 60mg/ml, *K. pneumonia* and *P. aeruginosa* showed resistans at 10mg/ml, 20mg/ml and 70mg/ml, *S. aureus* showed resistans at 20mg/ml, 30mg/ml, 40mg/ml. All the test Organisms were subseptible at 80mg/ml, 90mg/ml and 100mg/ml suggesting that the higher the concentration of the leaf extracts, the higher the antimicrobial activity as shown in Table 2.

As seen in Table 3, the least concentration of Plant extract that showed a cidal activity (MBC) was determined by sub-culturing the test organism from MIC test onto fresh solid medium and incubated for 24 hours. The MBC of the extract against *E.coli* and *S.aureus* was at 50mg/ml, that of *K. pneumonia* was at 30mg/ml and that of *P. aeruginosa* was at 60mg/ml.

The presence of zones of inhibition on the agar plates showed that the plant extract possesses antibacterial activity on the tested organisms which included both Gram positive and Gram negative organisms and this activity appears to be broad spectrum due to the inhibition of Gram positive and Gram negative organisms. Although the zones of inhibition were lower than that exhibited by the control drug (Ciprofloxacin) as seen in table 4b. This could be due to the fact that the plant extract is crude and contains other constituents that do not possess antibacterial property. Also the ability of the extract to diffuse through the gel may be hindered because of large molecules (stearic hindrance).

The highest zone of inhibition for all the test organisms was seen at the highest concentrations of the extract (100mg/ml) except for *K. pneumonia* which was seen at 90 mg/ml. However, *E. coli*, *K. pneumonia* and *P. aeruginosa* showed irregular growth inhibition by the extract which suggests that the growth of the organisms is only being depleted or inhibited at a fixed concentration. As for *E. coli*, higher inhibition zone (10mm) at 10mg/ml was seen than at 70mg/ml which showed inhibition zone of 6mm. However, in the case of *S. aureus*, the zone of inhibition increased with the increase in the concentration of the extract. From table 4, it is seen that the increase or decrease in concentration in proportion to the growth rate of the organism is sometimes directly or inversely based on each organism.

Conclusion

The Methanolic extract of *A. wilkesiana* leaves growing in Abraka; Delta State, Nigeria was found to have antibacterial activity on the selected bacterial isolates tested at varied concentration. This supports the traditional use of the leaves for treatment of ailments associated with these bacteria by the local people. It is therefore suggested that research be carried out on the toxicity of the plant in order to know the safety and toxicity of the plant and establish a safe dosage regimen since the infusion of the leaves is taken orally by local people for

treating gastrointestinal disturbances, eaten as vegetables for managing diabetes and as an enema for children apart from its topical use for treating dermatological disorders and lots more.

Recommendation

Based on this research, it is therefore recommended that medicinal plants like *A. wilkesiana* be used as cheap and readily available sources of antibacterial agent for developing countries. With further work on the isolation and characterization of the active ingredients in the crude extract of *A. wilkesiana*.

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